

## Evaluation of Histopathological Effects of Contaminated Unexpired Gentamicin and Penicillin G Injections on the Kidney Tissues of Juvenile Wistar Rats

**Takon Iquo Asu\* and Antai Sylvester Peter**

Microbiology Department, Faculty of Biological Sciences, University of Calabar, Calabar, Nigeria

**\*Corresponding Author:** Takon Iquo Asu, Microbiology Department, Faculty of Biological Sciences, University of Calabar, Calabar, Nigeria.

**Received:** February 11, 2019; **Published:** April 01, 2019

### Abstract

The histo-pathological effects of contaminated unexpired gentamicin and penicillin G injections on the kidney tissues of juvenile Wistar rats have been investigated using standard microbiological and histo-pathological techniques. A total of ten (10) samples (eight (8) drug samples and two (2) control drugs) were obtained from patent medicine stores in Calabar, Cross River State-Nigeria. Experimental animals were eight (8) juvenile Wistar rats which weighed between 80g and 150g. These animals were subjected to intramuscular drug treatment by injecting 2 mL of 360 mg of penicillin and 58 mg of gentamicin injections for 6 days at 12 hourly intervals. The animals were sacrificed after two days of drug administration. The mean total heterotrophic count ranged between  $3.80 \pm 0.35$  cfu/mL and  $1.53 \pm 0.26$  cfu/mL. There was a significant difference at  $p < 0.05$  as compared with control. Similarly, the mean coliform count was  $2.16 \pm 0.68$  cfu/mL and  $0.91 \pm 0.33$  cfu/mL. There was no mould isolate. Certain drug spoilage microorganisms isolated from the drug samples were identified as, *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Candida albicans*, *Staphylococcus aureus*, etc. The freeze-dried and heat fixed thin sections of Wistar rats' kidney were observed under the microscope using x10, x40 and x100 magnifications. Results obtained revealed marked necrosis and erosion of proximal borders especially the focal epithelium of proximal convoluted tubules (PCT) of the kidney, with the retention of cyto-architecture. Plasmolysis was observed in the epithelial cells of the distal convoluted tubules (DCT) when treated with contaminated unexpired gentamicin and penicillin g samples as compared with control. Glomerular hypoplasia and fibrosis of the Bowman's space occurred in the kidney when compared with the control. Similarly, interstitial oedema occurred in the medulla, leading to focal tubular fibrosis as a result of microbial activity. Interstitial hemorrhage which led to complete tubular necrosis with loss of defined tubular cyto-architecture was also observed. This effect made the tissue fibrous resulting in non-functional or shrunken kidney as compared to control. Results obtained from this work have raised serious health concerns, considering the risks posed by treatment of patients with contaminated drugs.

**Keywords:** Histopathological; Wistar Rat; Drug; Contamination

### Abbreviation

Cfu/mL: Colony Forming Unit/Millilitre; mL: Millilitre; PCT: Proximal Convoluted Tubule; DCT: Distal Convoluted Tubule; G: Gram; Mg: Milligram; AG: Acid and Gas

### Introduction

Pharmaceutical products are substances used in the prevention, treatment and diagnosis of diseases [1]. They are meant to be safe and potent during manufacture, storage and use [2]. Formulation of an elegant, efficacious, commercially acceptable drug, which is both stable and acceptable to the patient, may involve the use of a variety of ingredients [3]. This process may create conducive conditions for growth,

survival and even extensive replication of contaminated and spoilage microorganisms in the products [2]. The metabolic versatility of microorganisms is such that almost any formulation ingredient from simple sugars to complex organic molecules may undergo modification or degradation by these microorganisms, thus leading to spoilage of the product [4]. It had been reported that, the physical and chemical statuses of pharmaceutical products influence the type and extent of microbial spoilage to which the drug is susceptible to Takon., *et al* [3].

According to Ezejinidu., *et al.* [5], pharmaceutical products may be considered spoil, if, low levels of acutely pathogenic microorganisms or higher levels of opportunistic pathogens are present. Similarly, toxic metabolites have been shown to persist, long after the removal of microorganisms originally present or where detectable physical and chemical changes have occurred in the product [6]. Deterioration of the product had resulted in loss of potency or initiation of infection of infection in the user [7].

The outcome of using a contaminated product may vary from patient to patient, depending on the type and degree of contamination and route of administration [8]. The most serious effect of contamination had been the use of contaminated sterile injectables and eye drops, where generalized bacteraemia shock was reported and in some cases death of patients have been reported [9]. Pharmaceutical products of widely differing forms or statuses ranging from liquids, suspensions, creams, inhalers, tablets and powders are known to be susceptible to contamination with a variety of microorganisms ranging from true pathogens to a collection of opportunistic pathogens [10]. Similarly, products with more nutritious components such as creams and lotions or sweetened solutions with carbohydrates, amino acids, vitamins and often with liquids are also susceptible to deterioration [4].

According to Takon and Antai [11], majority of cases of medicament-related infections are most times not recognized or reported as such. Most times, medicament-borne infections could spread for some time, before it is diagnosed. Once diagnosed, the offending product should be withdrawn and subjected to quality analysis and subsequent investigation of the incident done retrospectively [6]. This research seeks to evaluate the histopathological effects of contaminated gentamicin and penicillin g injections on the kidney tissues of juvenile Wistar rats.

## Materials and Methods

A total of ten (10) unexpired drug samples, comprising of liquid gentamicin (4) and control (1) and penicillin g (4) and control (1) injections, were purchased from patent medicine stores in Calabar, Nigeria for the analyses. Different general purpose and selective media were used in order to ensure that a wide spectrum of heterotrophic organisms grew. Different reagents were also used.

### Microbiological examination of drug samples

Different aseptic bacteriological techniques were employed in processing of the drug samples. Only representative portions (1g or 1 ml) of the content of drug samples were used for the microbiological analysis. Powdered samples were analyzed according to the method described by Ozolua., *et al* [10]. Samples were diluted using sterile water for injection as diluent.

### Toxicity test

Toxicity of the drug forms used was determined using the method described by Hall [12].

**Animals:** Eight (8) juvenile Wistar rats weighing 80g and 150g were bred locally in Microbiology Department, University of Calabar, Calabar, Nigeria. The animals were kept two (2) per cage. These animals were fed daily with standard rat chow (Nigerian Livestock Feeds Plc, Nigeria) and 50cl tap water supplied to each cage. Light-dark cycle exposure was 12 hourly with temperature range from 18°C (night) to 32°C (daytime). Standard protocols for use of animals for toxicological experiments were used in handling the rats.

**Preparation and administration of drug samples:** Crystalline penicillin and gentamicin injections, purchased from Bez Pharmacy Calabar, Nigeria served as control, while contaminated unexpired samples were obtained from different patent medicine stores in Calabar metropolis. The active ingredients were Benzyl penicillin sodium BP 1,000,000i $\mu$  600 mg for penicillin injection and 80mg for gentamicin injection. Penicillin solution was prepared according to manufacturer's prescription. Penicillin powder was dissolved in 2 ml water for injection and administered intramuscularly two times daily for 6 days using sterile 2 ml syringe at 360 mg per kg rat body weight. Similarly, Gentamicin was administered at 58mg and the dose effects monitored at divided doses of 6h interval [13].

### Toxicity assessment

The locomotor activity and body weight of the animals were assessed for six days. These animals were observed daily for toxicological and pharmacological signs of lethargy, morbidity, mortality, food consumption rate, blood chemistry measurements and haematological indices. Locomotion was observed as described by Ozolua., *et al.* [10], Maisanaba., *et al.* [8] for 30 minutes each day using sensitive loco-

motor meter (40 fc, motron products, Sweden). Direct consumption by animal was observed and possible systemic shock. The animals were sacrificed after 2 days from the last drug administration. On the 6<sup>th</sup> day, the animals were anaesthetized with Bouin's fluid for 5 minutes and dissected longitudinally. Blood sample bottles were used to collect 5ml blood through carotid artery cannulation. Ten percent formaldehyde was used to wash the stomach and intestine, in preparation for examination for lesions, using a magnifying glass attached to a dissecting fluorescent lamp (thousand and one lamps, England). Histopathological sections of the kidney were prepared and used for examination of histological alterations and damages [12].

### Histopathological analysis

The drug treated kidney were freeze-dried and then fixed in wax. It was later cut into thin sections using a microtome machine. These sections were ready for histopathological examinations. These sections of the heat fixed kidney sections were viewed under the microscope using x10, x40 and x100 magnification lenses respectively. The images observed were snapped using specialized software (Motic Images plus 2.0) and made into slides [10].

### Results

| Sampling point        | Drug Status | Total No. of Samples Analysed | Mean Counts of Microorganisms (x10 <sup>4</sup> CFU/ml/mg) |                     |                 |                  |
|-----------------------|-------------|-------------------------------|--|---------------------|-----------------|------------------|
|                       |             |                               | Mean heterotrophic Count                                   | Mean Coliform Count | Mean Mold Count | Mean Yeast Count |
| Patent Medicine Store | Unexpired   | 10                            | 3.80 ± 0.35  | 2.16 ± 0.68         | 0.00±0.00       | 0.26 ± 0.14      |
|                       |             |                               | 1.53 ± 0.26  | 0.91 ± 0.33         | 0.00 ± 0.00     | 0.04 ± 0.02      |
|                       |             |                               | 2.02 ± 0.08  | 1.14 ± 0.05         | 0.00 ± 0.00     | 0.00             |

**Table 1:** Enumeration of microbial isolates from contaminated unexpired gentamicin and penicillin G injections from Patent Medicine Stores in Calabar, Nigeria.

Values are means ± standard deviation.

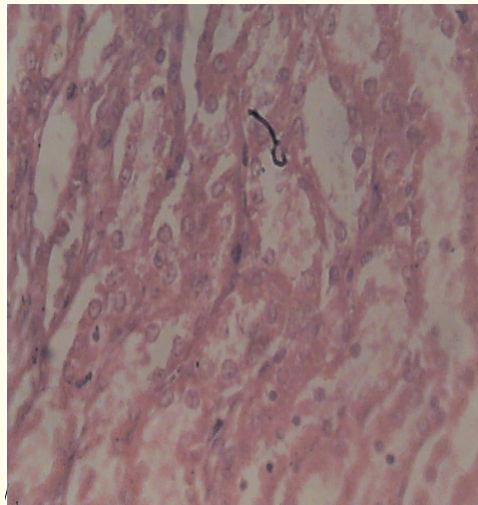
| Sample code | Cell Morphology   | Colonial Pigmentation      | Gram Stain | Catalase | Coagulase | Oxidase | Citrate utilization | Manitol | Glucose | Lactose | Urease | ONPG | Spore Formation | Voges Proskauer | Methyl red | Starch hydrolysis | Casein hydrolysis | Motility | Nitrate reductase | Indole production | Most probable organism/inference |
|-------------|-------------------|----------------------------|------------|----------|-----------|---------|---------------------|---------|---------|---------|--------|------|-----------------|-----------------|------------|-------------------|-------------------|----------|-------------------|-------------------|----------------------------------|
| 01          | Short rods        | Yellowish green            | -          | +        |           | +       | +                   | -       | -       | -       | -      | -    | -               | -               | +          | -                 |                   | +        | +                 | -                 | <i>Pseudomonas aeruginosa</i>    |
| 02          | Long rods         | White                      | +          | +        |           |         | +                   | -       |         |         |        |      | +               | +               |            | +                 | +                 |          |                   | -                 | <i>Bacillus subtilis</i>         |
| 03          | Short rods        | Red colonies or Mac-Conkey | -          | +        |           |         | -                   | +       | +       | +       | -      | +    |                 | -               | +          | -                 |                   | +        | +                 | +                 | <i>Escherichia coli</i>          |
| 04          | Cocci in clusters | Yellow or White Cream      | +          | +        | +         |         | +                   | + AG    | + AG    | + AG    | + AG   |      |                 |                 | +          |                   |                   |          | +                 |                   | <i>Staphylococcus aureus</i>     |

**Table 2a:** Biochemical characterization and identification of isolates from contaminated unexpired gentamicin and penicillin G injections from Patent Medicine stores in Calabar, Nigeria.

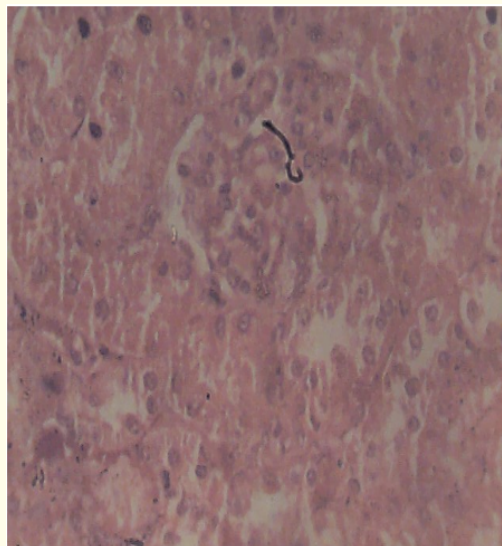
| Sample Code | Colonial Pigmentation | Subscription Colour | Nature of somatic hyphae | Nature of spores | Nature of production hyphae | Special vegetative | Budding | No. of spores | Vesicle shape | Gerim tube | Formation | Somatic nature | Type of productive spore | Growth on liquid (YEDP) | Medium | Most Probable Organism/Inference |
|-------------|-----------------------|---------------------|--------------------------|------------------|-----------------------------|--------------------|---------|---------------|---------------|------------|-----------|----------------|--------------------------|-------------------------|--------|----------------------------------|
| 01          | Creamy                | —                   | Pseudo hyphae            | Chlamydo-spores  |                             |                    | +       | Ascus Absent  | Oval          | +          |           | —              | Chlamydo-spores          | Film                    |        | <i>Candida albicans</i>          |
| 02          | Black                 | Brown               | Deptrate                 | Conidia          | Conidio-phores              | Foot cell          |         |               | Globose       | —          |           | Filament       | Conidia                  | —                       |        | <i>Aspergillus fumigatus</i>     |

**Table 2b:** Cultural, morphological and physiological characteristics of yeast/fungal isolates from contaminated unexpired gentamicin and penicillin g injection obtained from patent medicine stores.

Histopathological slides results of the thin sections of Wistar rat's kidney treated with contaminated unexpired gentamicin and Penicillin G injection for six (6) days and control



**Plate 1:** Showing kidney medulla (x40) treated with unexpired penicillin G injection. There is erosion of the medullary tubular epithelium with diffused medullar tissue necrosis.



**Plate 2:** Kidney cortex control (x40) shows renal tissues with normal glomerular with evenly dispersed cells. It was not treated with any antibiotics. This served as control.

## Discussion

The histopathological effects of contaminated unexpired gentamicin and penicillin G injections treatment on the kidney tissues of juvenile Wistar rats have been investigated using standard microbiological and histopathological techniques. A total of eight (8) juvenile Wistar rats and three (3) unexpired gentamicin and three (3) unexpired penicillin G injections obtained from patent medicine stores in Calabar, Nigeria were used for the research. The results of the mean counts of microbial isolates from unexpired drug samples as shown in table 1 revealed the highest mean heterotrophic count of  $3.8 \pm 0.55$  cfu/mL as compared to control for gentamicin injection, this result was significantly different at  $p < 0.05$ . The lowest mean heterotrophic count for contaminated gentamicin injection was recorded as  $1.53 \pm 0.26$  cfu/mL when compared with control. This result agrees with that reported by Ezejinidu., *et al.* [5], who observed that when drugs are not properly compounded could be contaminated by opportunistic microorganisms that may eventually spoil the drug. Similarly, the mean mold/coliform count was  $1.16 \pm 0.68$  cfu/mL and mean mold/yeast counts was  $0.26 \pm 0.33$ cfu/mL on nutrient agar for bacteria and sabouraud dextrose agar for fungi/yeast respectively. This result is in agreement with that reported by Denyer., *et al.* [2] and Takon., *et al.*

[3] where it was observed that certain microorganisms may contaminate and even grow and proliferate in drugs, where they degrade the active ingredients and use such as nutrient source. Similarly, the mean heterotrophic counts of isolates from unexpired penicillin G injection were  $2.02 \pm 0.08$  cfu/mL as compared to control. The mean coliform count was  $1.14 \pm 0.05$  cfu/mL and mean mold/yeasts count  $0.40 \pm 0.02$  cfu/mL on nutrient agar for bacteria and sabouraud dextrose agar for fungi/yeast respectively. These results were significantly different at  $p < 0.05$  when compared with control. The results obtained above is in agreement with that reported by Takon and Antai [4], where it was observed that drugs meant to be sterile during manufacture, storage and use, may be contaminated if there is a failure in the good manufacturing practice. Certain spoilage and pathogenic microorganisms were isolated from two drug samples of gentamicin and penicillin G injections. These organisms have also been isolated and identified by Takon and Antai [1], as drug spoilage organisms, which utilize the active ingredient of drugs as carbon source for nutrients. They have been shown to breakdown, proliferate in the drug products in the process spoil the drugs. These organisms have been reported as indicators of poor hygiene practices, pathogenic potential for route of administration and survival profile of these microorganisms and recoverability in the products.

The toxicological results revealed that the contaminated antibiotics had diverse toxic effects, which include weakness and numbness of the limbs, behavioural abnormalities. The contaminated unexpired antibiotics elicited mild neurotoxic effects at the site of the injection, at a dosage 360 mg per rat per day for penicillin G and 58 mg per rat per day for gentamicin. The results obtained differed from that obtained by Wihastuti, *et al.* [7], where it was reported that little or no toxicological effects were observed in rats after treatment with contaminated drugs. There was no significant difference in the haematological indices after treatment with contaminated antibiotics. These results agree with that reported by Tulsawani and Bhattacharya [6] where it was observed that, though drug contamination is a potential health hazard, there is little evidence for the production in pharmaceutical products of microbial toxins. No ulceration or other notable gastrointestinal lesions was observed in the drug treated rats on examination. This result supports the results obtained by Maisanaba, *et al* [8].

Histopathological effects of contaminated drugs treated kidney of Wistar rats revealed marked changes in the cyto-architecture, with necrosis of the proximal borders especially the focal epithelium of the proximal convoluted tubules (PCT) of the kidney. Epithelial cells of the distal convoluted tubules (DCT) were plasmolysed as a result of treatment with contaminated unexpired gentamicin injection samples from patent medicine stores. Results also showed fibrosis of the bowman's space and glomerula hypoplasia in the renal corpuscles of the kidney when compared with control. Also, microbial activity was reported to be responsible for medulla interstitial oedema which resulted in focal tubular fibrosis. This result has also been reported by Takon, *et al.* [3] that administration of contaminated parenteral could cause tissue damage or death in patients. Non-functional or shrunken kidney was observed, when compared to control upon treatment with contaminated unexpired gentamicin. This effect resulted in interstitial haemorrhage which led to complete tubular necrosis with loss of defined tubular cyto-architecture, thus making the tissues fibrous. On the whole all tubules were damaged, no inflammatory cells seen. These effects raised serious health concerns, considering the risk posed by contaminated drugs on patients. Similarly, kidney sections treated with contaminated unexpired penicillin G injection showed erosion of the medullary tubular epithelium and medullary tissue necrosis when compared to control.

Medicines are an essential part of human life and the safety of medicine is of utmost importance in providing the healthcare needs of patients. This work has shown that treatment of patients with contaminated drugs could lead to damage of tissues or even death of patients. The danger posed by treatment of patients with contaminated drugs cannot be over-emphasized.

## Conclusion

This research has shown that most sterile drugs could be contaminated when not properly compounded, stored or hygienically used. Treatment of patients with these contaminated drug products could be responsible for most medicament- borne infections in patients, which may lead to tissue or organ damage, and consequently death of the patients. The impact of drug contamination on the health of patients cannot be over-emphasised.

## Bibliography

1. Takon IA and Antai SP. "Microbial contamination of expired and unexpired aqueous and suspension drugs sold in some patent medicine stores in Calabar". *Nigerian Journal of Microbiology* 20.2 (2006): 1096-1103.



2. Denyer SP., *et al.* "Aspects of drug contamination". In: Hugo and Russell's Pharmaceutical Microbiology. 7<sup>th</sup> edition. Oxford, UK: Blackwell Science Publishing Co. (2004): 251-258.
3. Takon IA., *et al.* "Evaluation of toxicological effects of contaminated gentamicin injection on liver enzymes of juvenile wistar rats". *Journal of Medical Pharmaceuticals and Allied Sciences* 8 (2016): 132-141.
4. Iquo Asu Takon and Sylvester Peter Antai. "Evaluation of Histological Effects of Contaminated Unexpired Gentamicin Injections on the Kidney Tissues of Juvenile Wistar Rats". *Journal of Medical Science and Clinical Research* 6.8 (2018): 1010-1016.
5. Ezejindu DN., *et al.* "Evaluation of toxicological effects of carbon tetrachloride on liver". *International Journal of Scientific and Technology Research* 2.12 (2013): 372-374.
6. Tulsawani R and Bhattacharya R. "Effects of alpha-ketoglutarate on cyanide- induced biochemical alterations in rat brain and liver". *Biomedical and Environmental Sciences* 19.1 (2006): 61-66.
7. Wihastuti TA., *et al.* "Effects of subchronic exposure of PSP *Ganoderma lucidum* on renal function and histopathology features in *Rattus norvegicus* Wistar strain". *National Journal of Physiology, Pharmacy and Pharmacology* 5.4 (2015): 296-302.
8. Maisanaba S., *et al.* "In vivo evaluation of activities and expression of anti- oxidant enzymes in wistar rats exposed for 90 days to a modified clay". *Journal of Toxicology and Environmental Health* 77.8 (2014): 456-466.
9. Takon IA and Eyong EU. "Assessment of Microbial Quality of Selected Drug Samples Dispensed at Six Primary Health Centres in Aba Metropolis, Abia State-Nigeria". *British Journal of Pharmaceutical and Medical Research* 3.5 (2018).
10. Ozolua RI., *et al.* "Microbiological and toxicological studies on cellulose generated from agricultural wastes". *African Journal of Biotechnology* 4.10 (1996): 1147-1151.
11. Takon IA and Antai SP. "Effects of contaminated expired and unexpired penicillin g injection on haematological indices of juvenile wistar rats". *Journal of Microbiology and Biotechnology Research* 2.5 (2012): 750-757.
12. Hall RL. "Clinical Pathology of laboratory. Animals". In: *Animal Models in Toxicology* 2<sup>nd</sup> edition. New York: CRC Press (2007): 787-830.
13. *Guide to the Use and Care of Laboratory Animals National*. New York: Academy Press. 2101 Constitution Avenue (1996): 10-27.

**Volume 15 Issue 4 April 2019**

**©All rights reserved by Takon Iquo Asu and Antai Sylvester Peter.**